DHS SCIENCE AND TECHNOLOGY

Master Question List for COVID-19 (caused by SARS-CoV-2)

Weekly Report

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Homeland Security

Science and Technology

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FOREWORD

The Department of Homeland Security (DHS) is paying close attention to the evolving Coronavirus Infectious Disease (COVID-19) situation in order to protect our nation. DHS is working very closely with the Centers for Disease Control and Prevention (CDC), other federal agencies, and public health officials to implement public health control measures related to travelers and materials crossing our borders from the affected regions.

Based on the response to a similar product generated in 2014 in response to the Ebolavirus outbreak in West Africa, the DHS Science and Technology Directorate (DHS S&T) developed the following "master question list" that quickly summarizes what is known, what additional information is needed, and who may be working to address such fundamental questions as, "What is the infectious dose?" and "How long does the virus persist in the environment?" The Master Question List (MQL) is intended to quickly present the current state of available information to government decision makers in the operational response to COVID-19 and allow structured and scientifically guided discussions across the federal government without burdening them with the need to review scientific reports, and to prevent duplication of efforts by highlighting and coordinating research.

The information contained in the following table has been assembled and evaluated by experts from publicly available sources to include reports and articles found in scientific and technical journals, selected sources on the internet, and various media reports. It is intended to serve as a "quick reference" tool and should not be regarded as comprehensive source of information, nor as necessarily representing the official policies, either expressed or implied, of the DHS or the U.S. Government. DHS does not endorse any products or commercial services mentioned in this document. All sources of the information provided are cited so that individual users of this document may independently evaluate the source of that information and its suitability for any particular use. This document is a "living document" that will be updated as needed when new information becomes available.

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In general, PCR tests appear to be sensitive and specific, though confirmation of symptoms via chest CT is recommended. The sensitivity and specificity of serological testing methods is variable, and additional work needs to be done to determine factors that affect test accuracy.

Treatment for COVID-19 is primarily supportive care including ventilation if necessary.[128, 215] Numerous clinical trials are ongoing, but results are preliminary.[30,79] Several drugs show efficacy.

Remdesivir shows promise for reducing symptom duration in humans.[236]

Hydroxychloroquine is associated with elevated risk of cardiac arrhythmias and provides limited to no clinical benefit at this point in time.

Other pharmaceutical interventions are being investigated.

Additional clinical trial results are being released, and data from these trials are needed.

Work is ongoing to develop a SARS-CoV-2 vaccine in human and animal trials. No preliminary results are available.

Phase 2 Trials (initial testing for efficacy, continued testing for safety):

No preliminary vaccine results for humans have been released.

Broad-scale control measures such as stay-at-home orders are effective at reducing movement, and modeling shows evidence that they reduce transmission.

The effect of relaxing control measures is unknown, and research is needed to help plan for easing of restrictions.

As different US states have implemented differing control measures at various times, a comprehensive analysis of social distancing efficacy has not yet been conducted.

SARS-CoV-2 can persist on surfaces for at least 3 days and on the surface of a surgical mask for up to 7 days depending on conditions. If aerosolized intentionally, SARS-CoV-2 is stable for at least several hours. The seasonality of COVID-19 transmission is unknown.

Additional testing on SARS-CoV-2, as opposed to surrogate viruses, is needed to support initial estimates of stability.

Soap and water, as well as common alcohol and chlorine-based cleaners, hand sanitizers, and disinfectants are effective at inactivating SARS-CoV-2 on hands and surfaces.

Methods for decontaminating N95 masks have been approved by the FDA under an Emergency Use Authorization (EUA).

Additional decontamination studies, particularly with regard to PPE and other items in short supply, are needed.

The effectiveness of PPE for SARS-CoV-2 is currently unknown, and data from other related coronaviruses are used for guidance. Healthcare workers are at high risk of acquiring COVID-19, even with recommended PPE.

Most PPE recommendations have not been made on SARS-CoV-2 data, and comparative efficacy of different PPE for different tasks (e.g., intubation) is unknown. Identification of efficacious PPE for healthcare workers is critical due to their high rates of infection.

All current evidence supports the natural emergence of SARS-CoV-2 via a bat and possible intermediate mammal species. Identifying the intermediate species between bats and humans would aid in reducing potential spillover from a natural source. Wide sampling of bats, other wild animals, and humans is needed to address the origin of SARS-CoV-2.

Current evidence suggests that SARS-CoV-2 accumulates substitutions and mutations at a similar rate as other coronaviruses. Mutations and deletions in specific portions of the SARS-CoV-2 genome have not been linked to any changes in transmission or disease severity, though modeling work is attempting to identify possible changes.

Research linking genetic changes to differences in phenotype (e.g., transmissibility, virulence, progression in patients) is needed.

SARS-CoV-2 (COVID-19)	Infectious Dose – How much agent will make a healthy individual ill?
What do we know?	**The human infectious dose of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is unknown by all exposure routes. SARS-CoV-2 is the cause of coronavirus disease 19 (COVID-19).** *Work using SARS-CoV-2* • A total dose of approximately 700,000 plaque-forming units (PFU) of the novel coronavirus SARS-CoV-2 infected cynomolgus macaques via combination intranasal and intratracheal exposure (10^6 $TCID_{50}$ total dose).[274] Macaques did not exhibit clinical symptoms, but virus was shed from the nose and throat.[274] • Rhesus macaques are effectively infected with SARS-CoV-2 via the ocular conjunctival and intratracheal route at a dose of approximately 700,000 PFU (10^6 $TCID_{50}$).[96] • Rhesus macaques infected with 2,600,000 $TCID_{50}$ of SARS-CoV-2 by the intranasal, intratracheal, oral and ocular routes combined recapitulate moderate disease observed in the majority of human cases.[232] • Rhesus and cynomolgus macaques showed clinical infections at doses of 4.75 x 10^6 PFU (SARS-CoV-2 delivered through several routes), while common marmosets developed mild infections when exposed to 1.0 x 10^6 PFU intranasally.[204] • Ferrets infected with 316,000 $TCID_{50}$[163] or 600,000 $TCID_{50}$[267] of SARS-CoV-2 by the intranasal route show similar symptoms to human disease.[163, 267] Uninfected ferrets in direct contact with infected ferrets test positive and show disease as early as 2 days post-contact.[163] In one study, direct contact was required to transfer infection between ferrets,[163] however, transmission without direct contact was found in another study.[267] • Syrian Golden Hamsters infected with 100,000 PFU via the intranasal route closely resemble human respiratory infection. Uninfected hamsters in close contact with infected hamsters show symptoms within 4 days of exposure.[63] • Domestic cats exposed to 100,000 PFU of SARS-CoV-2 via the intranasal route developed severe pathological symptoms including lesions in the nose, throat, and lungs.[289] Younger cats exhibited more severe symptoms than older cats.[289] • Mice genetically modified to express the human ACE2 receptor were inoculated intranasally with 100,000 $TCID_{50}$ (~70,000 PFU) and developed pathological symptoms consistent with COVID-19.[26] **The correlation between the infectious dose and symptom severity is unknown.** • It has been suggested that higher viral loads at hospitalization correspond to more severe clinical outcomes,[200, 300] though pre- and asymptomatic individuals also show viral loads comparable to symptomatic individuals.[19, 366] *Related Coronaviruses* • The infectious dose for severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) in mice is estimated to be between 67-540 PFU (average 240 PFU, intranasal route).[93, 95] • Genetically modified mice expressing DPP4 exposed intranasally to doses of Middle East respiratory syndrome coronavirus (MERS-CoV) between 100 and 500,000 PFU show signs of infection. Infection with higher doses result in severe syndromes.[12, 80, 187, 358]
What do we need to know?	**Identifying the infectious dose for humans by the various routes through which we become infected is critical to the effective development of computational models to predict risk, develop diagnostics and countermeasures, and effective decontamination strategies. Animal studies are a plausible surrogate.** • Human infectious dose by aerosol route • Human infectious dose by surface contact (fomite) • Human infectious dose by fecal-oral route • Most appropriate animal model for SARS-CoV-2

SARS-CoV-2 (COVID-19)	Transmissibility – How does it spread from one host to another? How easily is it spread?
What do we know?	**SARS-CoV-2 is passed easily between humans, likely through close contact with relatively large droplets and possibly through smaller aerosolized particles.** • Pandemic COVID-19 has caused 4,222,968 infections and 287,809 deaths[153] in at least 187 countries and territories (as of 5/12/2020).[55, 284, 328] • In the US there are 1,354,504 confirmed COVID-19 cases across all 50 US states, with 81,076 deaths (as of 5/12/2020).[153] • Initial high-quality estimates of human transmissibility (R_0) range from 2.2 to 3.1.[213, 249, 270, 336, 357] • SARS-CoV-2 is believed to spread through close contact and droplet transmission,[59] with fomite transmission likely[155] and close-contact aerosol transmission plausible[41, 128] but unconfirmed.[327] • SARS-CoV-2 replicates in the upper respiratory tract, and infectious virus is detectable in throat and lung tissue for at least 8 days.[331] SARS-CoV-2 also infects human gut cell lines,[175] and ocular cells extracted from humans.[144] SARS-CoV-2 genetic material has been found in semen from both clinically symptomatic and recovered cases,[186] however, the infectiousness and the possibility of sexual transmission are unknown. • Contamination of patient rooms with aerosolized SARS-CoV-2 in the human respirable range (0.25-2.5 μm) indicates the potential for airborne transmission.[199] Viral RNA was detected up to 4 meters from ICU patient beds.[130] To date infectious virus has not been isolated from aerosol samples.[281] • SARS-CoV-2 may be spread by conversation and exhalation in the absence of cough, however more work is needed.[9, 185, 281] A preliminary study in China detailing a restaurant-associated outbreak supports aerosol transmission, though confirmation is needed.[192] • Experimentally infected ferrets were able to transmit SARS-CoV-2 to other ferrets by both direct contact (another ferret in same enclosure) as well as through the air (ferrets in an adjacent enclosure, separated by 10 cm).[267] • Evidence suggests that SARS-CoV-2 is not transmitted to infants during birth.[68, 72, 75, 283, 348, 350] **Individuals can transmit SARS-CoV-2 to others before they have symptoms.** • Pre-symptomatic[291, 360] or asymptomatic[24, 142, 209] patients can transmit SARS-CoV-2. At least 12% of all cases are estimated to be due to asymptomatic transmission.[101] It has been estimated that 23%,[197] 44%,[132] or as much as 56%[51] of infections may be caused by pre-symptomatic transmission. Infected patients transmit infections most often before symptoms began and within 5 days of symptom onset.[74] • Individuals may be infectious for 1-3 days prior to symptom onset,[317] and culturable virus has been found in individuals up to 6 days prior to symptom onset.[19] • Severe cases are more likely to transmit disease, and most new infections are within households of infected patients.[207] In China, it is estimated that infected individuals transmit COVID-19 to between 11.2%[34] and 16.3%[190] of household contacts, though even more may lack symptoms.[209] In New York, 38% of household contacts of infected patients became infected, with the proportion increasing with age (20% for contacts <5 years old, 55% for >65 years old).[275] **Undetected cases play a major role in transmission, and most cases are not reported.** • Models suggest up to 86% of early COVID-19 cases in China were undetected, and these infections were the source for 79% of reported cases.[189] • Models estimate that the true number of cases may be approximately 11 times greater than the reported number of cases in the UK,[349] and 5 to 10 times greater than the reported number of cases in the US.[156, 278] Preliminary estimates of the case reporting rate vary widely among countries, from roughly 1 reported case for every 3 actual cases (in Germany), to 1 in 149 (in China).[167]
What do we need to know?	**Identifying the contribution of asymptomatic or pre-symptomatic transmission is important for implementing control measures. Additionally, the relative contributions of different infection sources – fomites, droplets, aerosols, and potentially feces – are unknown.** • Capability of SARS-CoV-2 to be transmitted by contact with fomites (phones, doorknobs, surfaces, clothing, etc.) – see also Experimental Stability • Is sexual transmission possible? • Updated person to person transmission rates (e.g., R_0) as control measures take effect. • Is the R_0 estimate higher in healthcare or long-term care facilities? • When will infections peak in various cities and countries? • Are small-diameter (<5 μm) aerosol exposures capable of infecting humans? • How far do infectious aerosols (small-diameter, <5 μm) travel?

SARS-CoV-2 (COVID-19)	Host Range – How many species does it infect? Can it transfer from species to species?
What do we know?	**SARS-CoV-2 is closely related to other coronaviruses circulating in bats in Southeast Asia. Previous coronaviruses have passed through an intermediate mammal host before infecting humans, but the identity of the SARS-CoV-2 intermediate host is unknown.** • Early genomic analysis indicates similarity to SARS-CoV-1,[364] with a suggested bat origin.[82, 364] • Positive samples from the South China Seafood Market strongly suggests a wildlife source,[61] though it is possible that the virus was circulating in humans before the disease was associated with the seafood market.[29, 83, 342, 351] • Analysis of SARS-CoV-2 genomes suggests that a non-bat intermediate species is responsible for the beginning of the outbreak.[273] The identity of the intermediate host remains unknown.[191, 194-195] • Viruses similar to SARS-CoV-2 were present in pangolin samples collected several years ago.[174] **SARS-CoV-2 uses the same receptor for cell entry as the SARS-CoV-1 coronavirus that circulated in 2002/2003.** • Experiments show that SARS-CoV-2 Spike (S) receptor-binding domain binds the human cell receptor (ACE2) stronger than SARS-CoV-1,[334] potentially explaining its high transmissibility. The same work suggests that differences between SARS-CoV-2 and SARS-CoV-1 Spike proteins may limit the therapeutic ability of SARS antibody treatments.[334] • Modeling of SARS-CoV-2 Spike and ACE2 proteins suggests that SARS-CoV-2 can bind and infect human, bat, civet, monkey and swine cells.[308] Host range predictions based on structural modeling, however, are difficult,[117] and additional animal studies to better define the host range of SARS-CoV-2 are needed. • In vitro experiments suggest a broad host range for SARS-CoV-2, with more than 44 potential animal hosts, based on viral binding to species-specific ACE2 orthologs.[198] The host range is predicted to be limited primarily to mammals. • Genetic and protein analysis of primates suggests that African and Asian primates are likely more susceptible to SARS-CoV-2, while South and Central American primates are likely less susceptible,[225] confirming the SARS-CoV-2 host range is important for identifying animal reservoirs. • Changes in proteolytic cleavage of the Spike protein can also affect cell entry and animal host range, in addition to receptor binding.[226] **To date, ferrets, mink, hamsters, cats, and primates have been shown to be susceptible to SARS-CoV-2 infection. It is unknown whether these animals can transmit infection to humans.** • Animal model studies suggest that Golden Syrian hamsters, primates, and ferrets may be susceptible to infection.[63, 163] In the Netherlands, farmed mink developed breathing and gastrointestinal issues, which was diagnosed as SARS-CoV-2 infection.[2] • Domestic cats are susceptible to infection with SARS-CoV-2 (100,000 PFU via the intranasal route), and can transmit the virus to other cats via droplet or short-distance aerosol.[289] Dogs exposed to SARS-CoV-2 produced anti-SARS-CoV-2 antibodies but exhibited no clinical symptoms.[289] • Wild cats (tigers)[316] can be infected with SARS-CoV-2, although their ability to spread to humans is unknown.[214, 355] Two cases have been confirmed of pet domestic cats infected with SARS-CoV-2.[54] • Ducks, chickens, and pigs remained uninfected after experimental SARS-CoV-2 (30,000 CFU for ducks and chickens, 100,000 PFU for pigs, all via intranasal route).[289] There is currently no evidence that SARS-CoV-2 infects livestock,[149] though modeling suggests sheep, cows, pigs, and goats may be susceptible to infection by SARS-CoV-2.[173]
What do we need to know?	**Several animal models have been developed to recreate human-like illness, though to date they have been infected with high dose exposures. Lower dose studies may better replicate human disease acquisition.** • What is the intermediate host(s)? • What are the mutations in SARS-CoV-2 that allowed human infection and transmission? • What other animals can SARS-CoV-2 infect (e.g., potential wildlife reservoirs)? • Can infected animals transmit to humans (e.g., pet cats to humans)? • Can SARS-CoV-2 circulate in animal reservoir populations, potentially leading to future spillover events?

SARS-CoV-2 (COVID-19)	Incubation Period – How long after infection do symptoms appear? Are people infectious during this time?
What do we know?	**The majority of individuals develop symptoms within 14 days of exposure. For most people, it takes at least 2 days to develop symptoms, and on average symptoms develop 5 days after exposure. Incubating individuals can transmit disease for several days before symptom onset. Some individuals never develop symptoms but can still transmit disease.** • The best current estimate of the COVID-19 incubation period is 5.1 days, with 99% of individuals exhibiting symptoms within 14 days of exposure.[180] Fewer than 2.5% of infected individuals show symptoms sooner than 2 days after exposure.[180] • Individuals can test positive for COVID-19 even if they lack clinical symptoms.[24, 62, 129, 296, 360] • Individuals can be infectious while asymptomatic,[59, 276, 296, 360] and asymptomatic and pre-symptomatic individuals have similar amounts of virus in the nose and throat compared to symptomatic patients.[19, 162, 366] • Peak infectiousness may be during the incubation period, one day before symptoms develop.[132] Infectious virus has been cultured in patients up to 6 days before the development of symptoms.[19] • Infectious period is unknown, but possibly up to 10-14 days.[8, 189, 284] • Asymptomatic individuals are estimated to be infectious for a median of 9.5 days.[140] • On average, there are approximately 4[101] to 7.5[188] days between symptom onset in successive cases of a single transmission chain (i.e., the serial interval). Based on data from 339 transmission chains in China, the mean serial interval is 5.29 days.[100] • Children are estimated to shed virus for 15 days on average, with asymptomatic individuals shedding virus for less time (11 days) than symptomatic individuals (17 days).[206] • Most hospitalized individuals are admitted within 8-14 days of symptom onset.[362]
What do we need to know?	**The incubation period is well-characterized. Patients may be infectious, however, for days before symptoms develop.** • What is the average infectious period during which individuals can transmit the disease?

SARS-CoV-2 (COVID-19)	Clinical Presentation – What are the signs and symptoms of an infected person?
What do we know?	**Many COVID-19 cases are asymptomatic. Most symptomatic cases are mild, but severe disease can be found in any age group.[6] Older individuals and those with underlying medical conditions are at higher risk of serious illness and death.** • Approximately 18-31% of patients are asymptomatic throughout the course of their infection.[230, 238, 302] These estimates are based on studies that minimize the likelihood of including pre-symptomatic patients, which can obscure asymptomatic rates.[19] • The majority of symptomatic COVID-19 cases are mild (81%, n=44,000 cases).[296] Initial COVID-19 symptoms include fever (87.9% overall, but only 44-52% present with fever initially),[17, 129] cough (67.7%),[129], fatigue, shortness of breath, headache, and reduced lymphocyte count.[60, 65, 141] Chills, muscle pain, headache, sore throat, and loss of taste or smell[346] are also possible COVID-19 symptoms.[60] The prevalence of GI symptoms varies.[119] Neurological symptoms such as agitation and confusion may present with COVID-19,[133] and may be more common in severe cases.[88] Ocular issues[338] and skin lesions may also be symptoms of COVID-19.[38] • Complications include acute respiratory distress (ARDS, 17-29% of hospitalized patients, leading to death in 4-15% of cases),[71, 141, 310] pneumonia,[245] cardiac injury (20%),[290] secondary infection, kidney damage,[292] arrhythmia, sepsis, and shock.[129, 141, 310, 362] Most deaths are caused by respiratory failure or respiratory failure combined with myocardial (heart) damage.[277] A number of immunological indicators may help differentiate between severe and non-severe cases.[21, 104, 131, 143, 254, 294] • Approximately 15% of hospitalized patients are classified as severe,[129, 296] and approximately 5% of patients are admitted to the ICU.[129, 296] Patient deterioration can be rapid.[127] The survival rate of patients requiring mechanical ventilation varies widely (e.g., 35%,[148] 70%,[20] 75.5%[268]). • Clotting issues may be associated with severely ill COVID-19 patients[165] and those with ARDS.[88] COVID-19 patients should be monitored for possible thrombosis.[183] **The case fatality rate varies substantially by patient age and underlying comorbidities.** • Cardiovascular disease, hypertension, diabetes, and respiratory conditions all increase the CFR.[224, 296, 362] Hypertension and obesity are common in the US[119] and contribute to mortality.[18, 247] • Individuals >60 are at higher risk of death, and the CFR for individuals >85 is between 10 and 27%.[296, 362] In a small study, men exhibited more severe symptoms and died at higher rates than women.[154] The effect of comorbidities on the likelihood of severe symptoms is higher for men.[227] • Deaths due to COVID-19 are underreported. In New York City, up to 5,293 (22%) of period-specific excess deaths are unexplained and could be related to the pandemic.[240] More work is needed. **Additional studies on vulnerable subpopulations are required.** • African Americans are disproportionately represented in hospitalized populations (33% of hospitalized patients, only 18% of the base study population),[119] despite having similar rates of several underlying conditions as other groups.[126] • Pregnant women appear to develop severe symptoms at the same rate as the general population,[70, 159, 352] and current reports suggest no increase in risk of pre-term birth.[347] Most studies of COVID-19 in pregnancy represent women in later stages of pregnancy. **Children are susceptible to COVID-19,[99] though generally show milder[66, 205] or no symptoms.** • Between 21-28% of children may be asymptomatic.[205, 250, 257] A detailed study of 100 children with COVID-19 found that 21% were asymptomatic, 58% developed mild illness, 19% had moderate illness, 1% had severe illness, and 1% developed critical illness.[250] • Severe symptoms in children are possible[196] and more likely in those with complex medical histories.[285] Infant deaths have been recorded.[43, 205] • Early reports indicate the possibility of rare hyperinflammatory syndromes or shock in children (termed Pediatric Multi-System Inflammatory Syndrome[125]), putatively linked to COVID-19 infection.[271] Additional work is needed to confirm a link with COVID-19.
What do we need to know?	**The true case fatality rate is unknown, as the exact number of cases is uncertain. Testing priorities and case definitions vary by location. The proportion of asymptomatic infections is not known.** • How long does it take for infected individuals to recover outside of a healthcare setting? • What proportion of infected individuals are asymptomatic? Does this vary by age, location, or comorbidities? • Studies that test entire populations repeatedly over time and link those tests to the presence or absence of symptoms are necessary.

SARS-CoV-2 (COVID-19)	Protective Immunity – How long does the immune response provide protection from reinfection?
What do we know?	**Infected patients show productive immune responses, however more data is needed.** • In a limited study (n=9), hospitalized patients shed high levels of infectious virus for 7 days via the nasal-pharyngeal route, 50% of patients produced antibodies within 7 days, and all patients produced antibodies by 14 days. Antibody production did not correlate with lower viral load.[331] • In a larger study (n=175), most patients developed neutralizing antibodies within 10-15 days after disease onset. Elderly patients had significantly higher neutralizing antibody titers than younger patients.[335] In a separate study, elderly patients also showed higher viral loads than younger patients.[300] • In a study of 285 COVID-19 patients, 100% developed antiviral immunoglobulin-G within 19 days of symptom onset.[201] The neutralizing ability of these antibodies was not tested.[201] In a smaller in vitro study (n=23 patients), levels of antibodies (immunoglobulins M and G) were positively correlated with SARS-CoV-2 neutralizing ability.[300] • In a small series of 26 mild COVID-19 cases, researchers found prolonged persistence of SARS-CoV-2 antibodies and SARS-CoV-2 RNA for up to 50 days. Additionally, one patient cleared SARS-CoV-2 without developing a significant antibody response.[309] • Based on one patient, a productive immune response is generated and sustained for at least 7 days.[297] Previous studies on coronavirus immunity suggest that neutralizing antibodies may wane after several years.[46, 337] More data are needed. • A small subset of COVID-19 patients in China (8%) did not develop a serological response to infection, though the potential for reinfection in these patients is unknown.[335] Similarly, between 16.7% (for IgG) and 51.7% (for IgM) of patients in a separate study did not exhibit any immune response, in terms of production of those two types of antibodies.[295] • In a study of 221 COVID-19 patients, levels of two types of antibodies (IgM and IgG) were not associated with the severity of symptoms.[139] However, in a smaller study, patients with severe disease showed stronger antibody responses than those with non-severe symptoms.[300] • The early recovery phase of COVID-19 patients is characterized by inflammatory immune response,[319] suggesting the potential for adverse reactions after clinical improvement. **Currently, there is no evidence that recovered patients can be reinfected with SARS-CoV-2.** • Experimentally infected macaques were not capable of being reinfected after their primary infection resolved.[25] • According to the WHO, there is no evidence of re-infection with SARS-CoV-2 after recovery.[179] • Patients can test positive via PCR for up to 37 days after symptoms appear,[362] and after recovery and hospital discharge.[176] The ability of these individuals to infect others is unknown. **Similarly, there is no evidence that recovered patients are protected against reinfection with SARS-CoV-2.[324]** • Additional research is required before any conclusions can be drawn about the duration of protective immunity after SARS-CoV-2 infection.[13]
What do we need to know?	**Understanding the duration of protective immunity is limited by small sample sizes. Animal models are plausible surrogates. Additional research to quantify the risk of reinfection after weeks, months, and years is needed.** • How long does the immune response last? • Is there evidence of waning immunity? • Can humans become reinfected? • Are patients who test positive weeks after discharge from hospital capable of transmitting infection? • How does the patient immune response vary by age or disease severity? • How do different components of the immune response contribute to long-term protection?

SARS-CoV-2 (COVID-19)	Clinical Diagnosis – Are there tools to diagnose infected individuals? When during infection are they effective?
What do we know?	**Diagnosis relies on identifying the genetic signature of the virus in patient nose, throat, or sputum samples. These tests are relatively accurate. Confirmed cases are still underreported.** • The US CDC has expanded testing criteria to include symptomatic patients at clinician discretion.[28] • PCR protocols and primers have been widely shared internationally.[52, 85, 188, 288, 323, 329] PCR-based diagnostic assays are unable to differentiate between active and inactive virus. • A combination of pharyngeal (throat) RT-PCR and chest tomography is the most effective diagnostic criteria (correctly diagnoses 91.9% of infections).[265] A single throat swab detects 78.2% of infections, and duplicate tests identify 86.2% of infections.[265] • PCR tests using saliva were better able to detect SARS-CoV-2 RNA than those using nasopharyngeal swabs, and may be useful for self, at-home sampling.[340] Additional validation is needed. • Nasal and pharyngeal swabs may be less effective as diagnostic specimens than sputum and bronchoalveolar lavage fluid,[312] although evidence is mixed.[331] Combination RT-PCR and serology (antibody) testing may increase the ability to diagnose patients with mild symptoms, or identify patients at higher risk of severe disease.[359] • RT-PCR tests can identify asymptomatic cases; SARS-CoV-2 infection was identified in 2/114 individuals cleared by clinical assessment.[136] • The FDA issued an Emergency Use Authorization for an antigen-based diagnostic assay, limited to use in certified laboratories (Clinical Laboratory Improvement Amendments, CLIA).[106] • The FDA released an Emergency Use Authorization enabling laboratories to develop and use tests in-house for patient diagnosis.[110] • Tests from the US CDC are available to states.[52, 59] • Multiple rapid or real-time test kits have been produced by universities and industry, including the Wuhan Institute of Virology,[89] BGI,[33] Cepheid,[307] Abbot,[108] and Mesa Biotech.[35] • The US CDC is developing serological tests to determine what proportion of the population has been exposed to SARS-CoV-2.[158] A rapid antibody test by Cellex is now authorized by the FDA.[135, 320] • Home tests are being developed; however, none are FDA approved, nor are they useable as a diagnostic.[233-234, 248] • Artificial intelligence algorithms were able to improve the ability of radiologists to distinguish COVID-19 pneumonia from non-COVID-19 pneumonia on chest CT scans.[23] **Validated serological (antibody) assays are being developed to help determine who has been exposed to SARS-CoV-2. Serological evidence of exposure does not indicate immunity.** • Researchers found high specificity in a number of enzyme-linked immunosorbent assays (ELISA), though sample sizes for SARS-CoV-2 patients were small.[239] Additional research has shown high variability in the ability of tests (ELISA and lateral flow assays) by different manufacturers to accurately detect positive and negative cases (sensitivity and specificity, respectively).[178, 321] Lateral flow assays may be less reliable than ELISA.[10] • In one German town, serological testing has been used to identify the percent of the population already exposed to SARS-CoV-2 (14%), which can assist in public health response planning.[263] • Preliminary serological studies in Santa Clara and Los Angeles, California, estimated that 2.5-4.1% of the population has already been exposed to SARS-CoV-2 since the first confirmed cases in January,[31, 215] which is between 28 and 85 times greater than official reports. There are issues, however, with non-random study populations,[31] as well as false positive rates of the diagnostic tests themselves.[215] The false positive rate of the diagnostic assay used may account for a substantial portion of the reported infections,[31] particularly if the true proportion of positive patients is low. • In New York, initial serological testing indicates that 13.9% of the population has been exposed to COVID-19, approximately 10 times greater than the number of reported cases.[3] This is in line with other underreporting estimates in the US.[156, 278]
What do we need to know?	**In general, PCR tests appear to be sensitive and specific, though confirmation of symptoms via chest CT is recommended. The sensitivity and specificity of serological testing methods is variable, and additional work needs to be done to determine factors that affect test accuracy.** • How accurate are clinical diagnoses compared to genetic tests? • How effective are different swab specimens as diagnostic samples? • How many serological tests need to be done to obtain an accurate picture of underlying exposure?

SARS-CoV-2 (COVID-19)	Medical Treatments – Are there effective treatments?
What do we know?	**Treatment for COVID-19 is primarily supportive care including ventilation if necessary.[129, 216] Numerous clinical trials are ongoing, but results are preliminary.[30,79] Several drugs show efficacy.** • Two WHO-backed clinical trials (Solidarity and Discovery)[170] include remdesivir, hydroxychloroquine and chloroquine, ritonavir/lopinavir, and ritonavir/lopinavir and interferon-beta.[170] **Remdesivir shows promise for reducing symptom duration in humans.[237]** • Remdesivir can reduce the duration of symptoms in infected individuals, from 15 days to 11 days on average (compared to controls).[237] There is a possibility remdesivir may reduce mortality rates, though the result was not statistically significant.[237] In this trial, individuals with mild symptoms were excluded.[237] Remdesivir has received an Emergency Use Authorization from the FDA.[235] • In a clinical trial of severe COVID-19 patients, the effects of remdesivir were inconclusive due to a limitation in the study sample size.[314] For available patients, remdesivir did not reduce the time to recovery overall, but did show a tendency to reduce symptom duration for patients given the drug early.[314] This trial ended early, reducing its statistical power.[314] **Hydroxychloroquine is associated with elevated risk of cardiac arrhythmias and provides limited to no clinical benefit at this point in time.** • Several studies have found no benefit of hydroxychloroquine (with or without azithromycin) for reducing the need of intensive care,[122] reducing mortality,[211-212] or reducing the duration of symptoms.[69] Studies have documented the development of[212] or potential for cardiac arrhythmias in patients given hydroxychloroquine.[32, 228] One study suggested increased mortality rates in patients taking hydroxychloroquine alone.[211] Individuals taking hydroxychloroquine or colchicine for autoimmune disorders were not protected from COVID-19,[123] though sample sizes were limited. • Initial results purporting benefits of hydroxychloroquine and azithromycin[121] have been called into question by other researchers[146] and the journal's publishing society.[150] One small clinical trial (n=62) suggests that hydroxychloroquine can reduce recovery time compared to control group.[73] Key details are missing from this preprint.[73] **Other pharmaceutical interventions are being investigated.** • A randomized Phase II trial found that a triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin administered early in infection reduced symptom severity, viral shedding, and hospital stay time compared to patients taking lopinavir-ritonavir alone.[147] • Limited, preliminary evidence from clinical trials supports the efficacy of favipiravir[67] (which has been approved to treat COVID-19 in China)[1] and intravenous immunoglobulin.[48] Early research found no efficacy from combination ritonavir and lopinavir.[47] • Early results from a randomized clinical trial found that high doses (600 mg, twice per day) of chloroquine diphosphate were associated with toxicity and lethality in patients with severe COVID-19.[36] The trial did not have a large enough sample size to assess chloroquine treatment efficacy.[36] • A small (n=21), observational study found benefits of tocilizumab in severe COVID-19 patients.[343] Potential benefits of immunosuppressants[7] should be weighed against potential risks.[260] • Clinical trials are underway with famotidine[37] (a heartburn reliever) and mesenchymal stromal cells (cells derived from convalescent patient bone marrow)[138] to treat ARDS. • The anticoagulant heparin is being used to mitigate risks of pulmonary embolism, potentially caused by COVID-19-associated clotting problems.[104] An observational cohort study found that systemic anticoagulant use was associated with reduced mortality rates, particularly in patients requiring mechanical ventilation.[246] • Passive antibody therapy (convalescent serum)[49] is being given to patients (FDA Investigational New Drug approval).[109] In a small trial (n=5 patients),[286] convalescent sera administration was followed by clinical improvement.[286] Large efforts are underway to broaden and enhance serum therapies.[287] • Corticosteroids are commonly given to COVID-19 patients[362] at risk of ARDS,[344] but their use is not recommended by the US CDC.[56] A small (n=115 patients) trial in China found no benefit of corticosteroids in terms of admission to the ICU or death,[311] though a small study suggested that methylprednisolone can reduce hospital stay time.[313]
What do we need to know?	**Additional clinical trial results are being released, and data from these trials are needed.** • Are convalescent plasma treatments effective in humans or animals? • Do monoclonal antibodies exhibit any efficacy in human trials? • Are viral replicase inhibitors such as beta-D-N4-hydroxycytidine effective against SARS-CoV-2?[27]

SARS-CoV-2 (COVID-19)	Vaccines – Are there effective vaccines?
What do we know?	**Work is ongoing to develop a SARS-CoV-2 vaccine in human and animal trials. No preliminary results are available.** • Multiple entities are working to produce a SARS-CoV-2 vaccine,[14] including HHS/NIH/NIAID,[134, 184] CEPI, Moderna Therapeutics, Pfizer,[105] Gilead Sciences,[4-5, 236] Sanofi,[39] and Johnson and Johnson.[157] • Vaccine candidates undergoing clinical trial are listed below. *Phase 2 Trials (initial testing for efficacy, continued testing for safety)*: • China's CanSino is the first to complete Phase 1 safety trials of their adenovirus type5 vector based Sars-CoV-2 vaccine, Ad5-nCoV, and has advanced to Phase 2 human trials.[193] *Phase 1 Trials (initial testing for safety)*: • Sinovac Biotech has reported that their inactivated virus vaccine shows protective effects in rhesus macaques, particularly at high doses.[118] The vaccine is currently in phase 1 clinical trials.[81] Moderna has a Phase 1 trial underway based on its mRNA platform, mRNA-1273. • Inovio had their IND approved by the FDA and have started their Phase 1 clinical trials on their DNA vaccine candidate INO-4800.[279] • Shenzhen Geno-Immune Medical Institute is testing its aAPC[222] and lentiviral[221] vaccines in Phase 1 clinical trials. • BioNTech and Pfizer's BNT162 program is in Phase 1/2 clinical trial for four of its mRNA vaccine candidates.[253] • University of Oxford's ChAdOx1 vaccine is in Phase 1 clinical trials. This vaccine is based on a chimpanzee adenovirus expressing SARS-CoV-2 proteins.[252] • The Beijing Institute of Biological Products/Wuhan Institute of Biological Products have initiated a Phase I trial of their inactivated vaccine candidate.[330] • Symvivo Corporation has received approval to begin a Phase I trial with their oral bacTRL-Spike vaccine candidate in Canada.[219] • Novavax is testing a recombinant spike protein nanoparticle vaccine in Phase I trials.[220] • Immunitor LLC is starting Phase I trials of a heat-inactivated vaccine derived from pooled patient plasma.[223] *Co-opting existing vaccines* • Some efforts have begun to enroll healthcare workers in clinical trials to study the efficacy of the BCG (Bacillus Calmette-Guérin) vaccine for reducing symptom severity in COVID-19 patients.[218]
What do we need to know?	**No preliminary vaccine results for humans have been released.** • Safety of candidate vaccines in humans and animals • Efficacy of candidate vaccines in humans and animals • Length of any vaccine-derived immunity • Evidence for vaccine-derived enhancement (immunopotentiation)

SARS-CoV-2 (COVID-19)	Non-pharmaceutical Interventions – Are public health control measures effective at reducing spread?
What do we know?	**Broad-scale control measures such as stay-at-home orders are effective at reducing movement, and modeling shows evidence that they reduce transmission.** • Social distancing and other policies are estimated to have reduced COVID-19 spread by 44% in Hong Kong[87] and reduced spread throughout China[166, 169, 172, 202] and Italy.[120] Restrictive lockdowns in China are estimated to have reduced disease transmission within only a few days,[365] in part, through reductions in an individual's average number of contacts.[353] • Modeling demonstrates that multifaceted restrictions and quarantines in China reduced the R_0 of SARS-CoV-2 from greater than 3 to less than 1 between January 23 and February 5.[244] Additionally, movement restrictions and other control measures helped limit the amount of time where community transmission was possible (i.e., $R_0 > 1$).[354] • Models indicate that a combination of school closures, work restrictions, and other measures are required to effectively limit transmission.[113] School closures alone appear insufficient.[151, 172] • Non-pharmaceutical interventions in China did not reduce transmission equally across all groups; transmission rates in younger individuals, particularly infants, as well as hospital workers continued to increase even while overall transmission rates declined.[244] • Anonymized cell phone location data indicate major reductions in mobility due to social distancing.[115] Mobility in major US cities declined after each public health intervention implemented.[177] Survey data from the UK suggest large reductions in individuals' physical contacts after public health restrictions were put into effect.[152] • Contact tracing to identify infected individuals reduces the amount of time infectious individuals can transmit disease in a population and increases the time between successive cases (the serial interval).[34] • In a prospective study, researchers found that a larger number of public health interventions in place was strongly associated with lower COVID-19 growth rates in the next week.[160] **The effect of relaxing control measures is unknown, and research is needed to help plan for easing of restrictions.** • Modeling indicates that COVID-19 is likely to become endemic in the US population, with regular, periodic outbreaks, and that additional social or physical distancing measures may be required for several years to keep cases below critical care capacity in absence of a vaccine or effective therapeutic.[164] Results depend critically on the duration of immunity after exposure.[164] • Modeling suggests that premature lifting of social distancing measures will substantially increase the number of local COVID-19 cases in Wuhan, China.[255] Similarly, forecasts in the US estimate a resumption of exponential case growth if social distancing measures are relaxed.[90] • In the UK, modelers are assessing the efficacy of rolling interventions, whereby social distancing measures are put into place every few weeks to keep healthcare demand below a critical point.[349] • A modeling study using Chinese data suggests that carefully balancing control measures to maintain R_0 below 1 would be more efficient than allowing R_0 to increase above 1 at any point.[181] • Robust contact tracing and case finding may be needed to control COVID-19 in the US, but would require additional staff and resources to conduct effectively.[315] • A model suggests that reducing public contacts is more important for controlling spread than reducing private (e.g., in-home) contacts.[217] • The WHO has released guidelines on public health strategy, focusing on controlling transmission, ensuring public health capacity is robust, and engaging local communities.[322] • Johns Hopkins released a report outlining how to re-open certain categories of activities (e.g., schools, restaurants, events) while reducing COVID-19 risk; the report also ranks certain activities by their contact intensity, number of contacts, and the potential to modify them to reduce risk.[272] • SARS-CoV-2 levels in wastewater may track with prevalence in the population,[339] and could be used to monitor viral elimination in an area.
What do we need to know?	**As different US states have implemented differing control measures at various times, a comprehensive analysis of social distancing efficacy has not yet been conducted.** • How many cases in the US have been averted due to social distancing restrictions? • What are plausible options for relaxing social distancing and other intervention measures without resulting in a resurgence of COVID-19 cases? • How is COVID-19 incidence changing in states that have begun easing movement and activity restrictions?

<table>
<tr><th>SARS-CoV-2 (COVID-19)</th><th>Environmental Stability – How long does the agent live in the environment?</th></tr>
<tr><td>What do we know?</td><td>SARS-CoV-2 can persist on surfaces for at least 3 days and on the surface of a surgical mask for up to 7 days depending on conditions. If aerosolized intentionally, SARS-CoV-2 is stable for at least several hours. The seasonality of COVID-19 transmission is unknown.
SARS-CoV-2 Data
• SARS-CoV-2 can persist on plastic and metal surfaces between 3 days (21-23°C, 40% RH)[304] and 7 days (22°C, 65% RH). Infectious virus can be recovered from a surgical mask after 7 days (22°C, 65% RH).[77]
• SARS-CoV-2 has an aerosol half-life of 2.7 hours (particles <5 µm, tested at 21-23°C and 65% RH).[304]
• SARS-CoV-2 is susceptible to heat treatment (70°C) but can persist for at least two weeks at refrigerated temperatures (4°C).[77, 262]
• SARS-CoV-2 genetic material (RNA) was detected in symptomatic and asymptomatic cruise ship passenger rooms up to 17 days after cabins were vacated. The infectiousness of this material is not known.[231]
• In a preliminary study, SARS-CoV-2 stability was enhanced when present with bovine serum albumin, which is commonly used to represent sources of protein found in human sputum.[251]
• No strong evidence exists showing a reduction in transmission with seasonal increase in temperature and humidity.[208] Modeling suggests that even accounting for potential reductions in transmission due to weather and behavioral changes, public health interventions will still need to be in effect to limit COVID-19 transmission.[229]
Surrogate Coronavirus data:
• Studies suggest that other coronaviruses can survive on non-porous surfaces up to 9-10 days (MHV, SARS-CoV),[50, 64] and porous surfaces for up to 3-5 days (SARS-CoV)[103] in air conditioned environments (20-25°C, 40-50% RH).
• Coronavirus survival tends to be higher at lower temperatures and lower relative humidity (RH),[50, 64, 259, 305] though infectious virus can persist on surfaces for several days in typical office or hospital conditions.[305]
• SARS can persist with trace infectivity for up to 28 days at refrigerated temperatures (4°C) on surfaces.[50]
• One hour after aerosolization approximately 63% of airborne MERS virus remained viable in a simulated office environment (25°C, 75% RH).[256]
• Porous hospital materials, including paper and cotton cloth, maintain infectious SARS-CoV for a shorter time than non-porous material.[171]</td></tr>
<tr><td>What do we need to know?</td><td>Additional testing on SARS-CoV-2, as opposed to surrogate viruses, is needed to support initial estimates of stability.
• Stability of SARS-CoV-2 in aerosol, droplets, and other matrices (mucus/sputum, feces)
• Particle size distribution (e.g., droplet, large droplet, and true aerosol distribution)
• Duration of SARS-CoV-2 infectivity via fomites and surfaces (contact hazard)
• Stability of SARS-CoV-2 on PPE (e.g., Tyvek, nitrile, etc.)
• Evidence for seasonality in transmission, or other environmental impacts (UV, temperature, humidity)</td></tr>
</table>

SARS-CoV-2 (COVID-19)	Decontamination – What are effective methods to kill the agent in the environment?
What do we know?	**Soap and water, as well as common alcohol and chlorine-based cleaners, hand sanitizers, and disinfectants are effective at inactivating SARS-CoV-2 on hands and surfaces.** *SARS-CoV-2* • Alcohol-based hand rubs are effective at inactivating SARS-CoV-2.[168] • Chlorine bleach (1%, 2%), 70% ethanol and 0.05% chlorhexidine are effective against live virus in lab tests.[76] • Twice-daily cleaning with sodium dichloroisocyanurate decontaminated surfaces in COVID-19 patient hospital rooms.[241] • EPA has released a list of SARS-CoV-2 disinfectants, but solutions were not tested on live virus.[11] *Other Coronaviruses* • Chlorine-based[326] and ethanol-based[84] solutions are recommended. • Heat treatment (56ºC) is sufficient to kill coronaviruses,[259, 361] though effectiveness depends partly on protein in the sample.[259] • 70% ethanol, 50% isopropanol, sodium hypochlorite (0.02% bleach), and UV radiation can inactivate several coronaviruses (MHV and CCV).[280] • Ethanol-based biocides effectively disinfect coronaviruses dried on surfaces, including ethanol containing gels similar to hand sanitizer.[145, 332] • Surface spray disinfectants such as Mikrobac, Dismozon, and Korsolex are effective at reducing infectivity of the closely related SARS-CoV-1 after 30 minutes of contact.[258] • Coronaviruses may be resistant to heat inactivation for up to 7 days when stabilized in stool.[298-299] • Coronaviruses are more stable in matrixes such as respiratory sputum.[102] **Methods for decontaminating N95 masks have been approved by the FDA under an Emergency Use Authorization (EUA).** • Researchers have identified four methods capable of decontaminating N95 respirators while maintaining physical integrity (fit factor): UV radiation, heating to 70ºC, and vaporized hydrogen peroxide (VHP).[114] Ethanol (70%) was associated with loss of physical integrity.[114] • Hydrogen peroxide vapor (VHP) can repeatedly decontaminate N95 respirators.[269] Devices capable of decontaminating 80,000 masks per day have been granted Emergency Use Authorization from the FDA.[106] • The FDA has issued an Emergency Use Authorization for a system capable of decontaminating 10 N95 masks at a time using devices already present in many US hospitals.[40]
What do we need to know?	**Additional decontamination studies, particularly with regard to PPE and other items in short supply, are needed.** • What is the minimal contact time for disinfectants? • Does contamination with human fluids/waste alter disinfectant efficacy profiles? • How effective is air filtration at reducing transmission in healthcare, airplanes, and public spaces? • Are landfills and wastewater treatment plants effective at inactivating SARS-CoV-2? • Is heat or UV decontamination effective to clean N95 masks, respirators and other types of PPE for multi-use?

SARS-CoV-2 (COVID-19)	PPE – What PPE is effective, and who should be using it?
What do we know?	**The effectiveness of PPE for SARS-CoV-2 is currently unknown, and data from other related coronaviruses are used for guidance. Healthcare workers are at high risk of acquiring COVID-19, even with recommended PPE.** • Healthcare worker illnesses[296] demonstrates human-to-human transmission despite isolation, PPE, and infection control.[282] • Risk of transmission to healthcare workers appears high, with up to 20% of healthcare workers in Lombardy, Italy becoming infected.[264] • Over 50% of US healthcare workers infected with COVID-19 report work in a healthcare setting as their single source of exposure.[44] • "Healthcare personnel entering the room [of SARS-CoV-2 patients] should use standard precautions, contact precautions, airborne precautions, and use eye protection (e.g., goggles or a face shield)."[57] • WHO indicates healthcare workers should wear clean long-sleeve gowns as well as gloves.[325] • Using a fluorescent simulant, researchers found contamination on exposed skin on healthcare workers while performing intubations.[112] Clothing and PPE that covers all skin may reduce exposure to pathogens.[112, 318] • Respirators (NIOSH-certified N95, EUFFP2 or equivalent) are recommended for those dealing with possible aerosols.[326] Additional protection, such as a Powered Air Purifying Respirator (PAPR) with a full hood, should be considered for high-risk procedures (i.e., intubation, ventilation).[42] • Particular attention should be paid to the potential for transmission via exhaled air during supportive respiratory procedures.[128] • There is evidence both for[199] and against[241] the detection of SARS-CoV-2 RNA via air sampling in patient rooms and other hospital areas. • Research at Johns Hopkins Center for Health Security has provided initial estimates of PPE needs in the US: 7.8 billion gloves, 668 million gowns, 360 million surgical masks, and 136 million N95 or similar respirators.[301] • KN95 respirators are, under certain conditions, approved for use under FDA Emergency Use Authorization.[107] On May 7, the FDA rescinded a number of KN95 models that no longer meet the EUA criteria and are no longer authorized.[111] **Masks may be effective at slowing transmission.** • Surgical face masks, respirators and homemade face masks may prevent transmission of coronaviruses from infectious individuals (with or without symptoms) to other individuals.[92, 182, 303] Surgical masks were associated with a significant reduction in the amount of seasonal coronavirus (not SARS-CoV-2) expressed as aerosol particles (<5 µm) compared to not wearing a mask.[182] Other preliminary work has failed to document protective efficacy of surgical or cotton masks,[22] and more SARS-CoV-2 specific research is needed. • On 4/3/2020, the US CDC recommended wearing cloth face masks in public where social distancing measures are difficult to maintain.[58] • The efficacy of homemade PPE, made with T-shirts, bandanas, or similar materials, is less than standard PPE, but may offer some protection if no other options are available.[78, 91, 266]
What do we need to know?	**Most PPE recommendations have not been made on SARS-CoV-2 data, and comparative efficacy of different PPE for different tasks (e.g., intubation) is unknown. Identification of efficacious PPE for healthcare workers is critical due to their high rates of infection.** • What is the importance of aerosol transmission (particles <5µm)? What is the effective distance of spread via droplet or aerosol? • How effective are barriers such as N95 respirators or surgical masks for SARS-CoV-2? • What is the appropriate PPE for first responders? Airport screeners? • What are proper procedures for reducing spread and transmission rates in medical facilities? • How effective are homemade masks at reducing SARS-CoV-2 transmission?

SARS-CoV-2 (COVID-19)	Forensics – Natural vs intentional use? Tests to be used for attribution.
What do we know?	**All current evidence supports the natural emergence of SARS-CoV-2 via a bat and possible intermediate mammal species.** • Genomic analysis places SARS-CoV-2 into the beta-coronavirus clade, with close relationship to bat coronaviruses. The SARS-CoV-2 virus is distinct from SARS-CoV-1 and MERS viruses.[98] • Genomic analysis suggests that SARS-CoV-2 is a natural variant and is unlikely to be human-derived or otherwise created by "recombination" with other circulating strains of coronavirus.[15, 364] • Genomic data support at least two plausible origins of SARS-CoV-2: "(i) natural selection in a non-human animal host prior to zoonotic transfer, and (ii) natural selection in humans following zoonotic transfer."[15] Both scenarios are consistent with the observed genetic changes found in all known SARS-CoV-2 isolates. • Some SARS-CoV-2 genomic evidence indicates a close relationship with pangolin coronaviruses,[333] and data suggest that pangolins may be a natural host for beta-coronaviruses.[194-195] Genomic evidence suggests a plausible recombination event between a circulating coronavirus in pangolins and bats could be the source of SARS-CoV-2.[341] Emerging studies are showing that bats are not the only reservoir of SARS-like coronaviruses.[356] Additional research is needed. • A novel bat coronavirus (RmYN02) has been identified in China with an insertion in the viral furin cleavage site. While distinct from the insertion in SARS-CoV-2, this evidence shows that such insertions can occur naturally.[363] • Additionally, "[…] SARS-CoV-2 is not derived from any previously used virus backbone," reducing the likelihood of laboratory origination,[15] and "[…] genomic evidence does not support the idea that SARS-CoV-2 is a laboratory construct, [though] it is currently impossible to prove or disprove the other theories of its origin."[15] • Work with other coronaviruses has indicated that heparan sulfate dependence can be an indicator of prior cell passage, due to a mutation in the previous furin enzyme recognition motif.[94]
What do we need to know?	**Identifying the intermediate species between bats and humans would aid in reducing potential spillover from a natural source. Wide sampling of bats, other wild animals, and humans is needed to address the origin of SARS-CoV-2.** • What tests for attribution exist for coronavirus emergence? • What is the identity of the intermediate species? • Are there closely related circulating coronaviruses in bats or other animals with the novel PRRA cleavage site found in SARS-CoV-2?

SARS-CoV-2 (COVID-19)	Genomics – How does the disease agent compare to previous strains?
What do we know?	**Current evidence suggests that SARS-CoV-2 accumulates substitutions and mutations at a similar rate as other coronaviruses. Mutations and deletions in specific portions of the SARS-CoV-2 genome have not been linked to any changes in transmission or disease severity, though modeling work is attempting to identify possible changes.** • There have been no documented cases of SARS-CoV-2 prior to December 2019. Preliminary genomic analyses, however, suggest that the first human cases of SARS-CoV-2 emerged between 10/19/2019 – 12/17/2019.[16, 29, 261] • Analysis of more than 7,000 SARS-CoV-2 genome samples provides an estimated mutation rate of 6×10^{-4} nucleotides per genome per year.[306] The same analysis estimates the emergence of SARS-CoV-2 in humans between October and December 2019.[306] This aligns with the first known human cases in China in early December 2019 and in Europe in late December 2019.[97] • There is currently no evidence of distinct SARS-CoV-2 phenotypes at this time.[210, 306] • Despite evidence of genomic areas under positive selection, there is no known association between particular mutations and changes in transmission or virulence.[45] • Pangolin coronaviruses are closely related to both SARS-CoV-2 and closely related bat coronaviruses. Phylogenetic analysis suggests that SARS-CoV-2 is of bat origin, but is closely related to pangolin coronavirus.[194-195] • The SARS-CoV-2 Spike protein, which mediates entry into host cells and is the major determinant of host range, is very similar to the SARS-CoV-1 Spike protein.[203] The rest of the genome is more closely related to two separate bat [203] and pangolin[195] coronaviruses. • An analysis of SARS-CoV-2 sequences from Singapore has identified a large nucleotide (382 bp) deletion in ORF-8.[293] In Arizona, researchers identified an 81-base pair deletion (removing 27 amino acids) in the ORF-7a protein, indicating that mutations can be detected by routine sentinel surveillance. The function of these deletions are unknown at this time.[137] • A recent report of virus mutations within patients needs more research.[161] Additional analysis of data suggests the results may be due to experimental methods.[124, 345] • Structural modeling suggests that observed changes in the genetic sequence of the SARS-CoV-2 Spike protein may enhance binding of the virus to human ACE2 receptors.[242] More specifically, changes to two residues (Q493 and N501) are linked with improving the stability of the virus-receptor binding complex.[242] Additionally, structural modeling identified several existing mutations that may enhance the stability of the receptor binding domain, potentially increasing binding efficacy.[243] Infectivity assays are needed to validate the genotypic changes and possible phenotypic results identified in these studies. • A key difference between SARS-CoV-2 and other beta-coronaviruses is the presence of a polybasic furin cleavage site in the Spike protein (insertion of a PRRA amino acid sequence between S1 and S2).[86] • The US CDC is launching a national genomics consortium to assess SARS-CoV-2 genomic changes over time.[53] • Preliminary phylogenetic analysis indicates at least three major SARS-CoV-2 clades that represent broad regions, though this is likely to change as additional genomes are sampled.[116] To date, there are no known differences in disease severity due to infection with different strains of SARS-CoV-2.
What do we need to know?	**Research linking genetic changes to differences in phenotype (e.g., transmissibility, virulence, progression in patients) is needed.** • Are there similar genomic differences in the progression of coronavirus strains from bat to intermediate species to human? • Are there different strains or clades of circulating virus? If so, do they differ in virulence?

Table 1. Definitions of commonly-used acronyms

Acronym/Term	Definition	Description
ACE2	Angiotensin-converting enzyme 2	Acts as a receptor for SARS-CoV and SARS-CoV-2, allowing entry into human cells
Airborne transmission	Aerosolization of infectious particles	Aerosolized particles can spread for long distances (e.g., between hospital rooms via HVAC systems). Particles generally <5 µm.
ARDS	Acute respiratory distress syndrome	Leakage of fluid into the lungs which inhibits respiration and leads to death
Attack rate	Proportion of "at-risk" individuals who develop infection	Defined in terms of "at-risk" population such as schools or households, defines the proportion of individuals in those populations who become infected after contact with an infectious individual
CCV	Canine coronavirus	Canine coronavirus
CFR	Case Fatality Rate	Number of deaths divided by confirmed patients
CoV	Coronavirus	Virus typified by crown-like structures when viewed under electron microscope
COVID-19	Coronavirus disease 19	Official name for the disease caused by the SARS-CoV-2 virus.
Droplet transmission	Sneezing, coughing	Transmission via droplets requires relatively close contact (e.g., within 6 feet)
ELISA	Enzyme-linked immunosorbent assay	Method for serological testing of antibodies
Fomite	Inanimate vector of disease	Surfaces such as hospital beds, doorknobs, healthcare worker gowns, faucets, etc.
HCW	Healthcare worker	Doctors, nurses, technicians dealing with patients or samples
Incubation period	Time between infection and symptom onset	Time between infection and onset of symptoms typically establishes guidelines for isolating patients before transmission is possible
Infectious period	Length of time an individual can transmit infection to others	Reducing the infectious period is a key method of reducing overall transmission; hospitalization, isolation, and quarantine are all effective methods
Intranasal	Agent deposited into external nares of subject	Simulates inhalation exposure by depositing liquid solution of pathogen/virus into the nose of a test animal, where it is then taken up by the respiratory system.
MERS	Middle-East Respiratory Syndrome	Coronavirus with over 2,000 cases in regional outbreak since 2012
MHV	Mouse hepatitis virus	Coronavirus surrogate
Nosocomial	Healthcare- or hospital-associated infections	Characteristic of SARS and MERS outbreaks, lead to refinement of infection control procedures
PCR	Polymerase chain reaction	PCR (or real-time [RT] or quantitative [Q] PCR) is a method of increasing the amount of genetic material in a sample, which is then used for diagnostic testing to confirm the presence of SARS-CoV-2
PFU	Plaque forming unit	Measurement of the number of infectious virus particles as determined by plaque forming assay. A measurement of sample infectivity.
PPE	Personal protective equipment	Gowns, masks, gloves, and any other measures used to prevent spread between individuals
R_0	Basic reproduction number	A measure of transmissibility. Specifically, the average number of new infections caused by a typical infectious individual in a wholly susceptible population.

Acronym/Term	Definition	Description
SARS	Severe Acute Respiratory Syndrome	Coronavirus with over 8,000 cases in global 2002-2003 outbreak
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2	Official name for the virus previously known as 2019-nCoV.
Serial interval	Length of time between symptom onset of successive cases in a transmission chain	The serial interval can be used to estimate R_0, and is useful for estimating the rate of outbreak spread
Superspreading	One individual responsible for an abnormally large number of secondary infections	Superspreading can be caused by high variance in the distribution of secondary cases caused by a single individual; most individuals infect very few people, while some infect a large number, even with the same average number of secondary infections
$TCID_{50}$	50% Tissue Culture Infectious Dose	The number of infectious units which will infect 50% of tissue culture monolayers. A measurement of sample infectivity.
Transgenic	Genetically modified	In this case, animal models modified to be more susceptible to MERS and/or SARS by adding proteins or receptors necessary for infection

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